Multigene kinase network, kidney transport, and salt in essential hypertension

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Evidence is mounting that a multi-gene kinase network is central to the regulation of renal Na^+ and K^+ excretion and that aberrant signaling through the pathway can result in renal sodium retention and hypertension (HTN). The kinase network minimally includes the Ste20-related proline–alanine-rich kinase (SPAK), the with-no-lysine kinases (WNKs), *WNK4* and *WNK1*, and their effectors, the thiazide-sensitive NaCl cotransporter and the potassium secretory channel, ROMK. Available evidence indicates that the kinase network normally functions as a switch to change the mineralocorticoid hormone response of the kidney to either conserve sodium or excrete potassium, depending on whether aldosterone is induced by a change in dietary sodium or potassium. Recently, common genetic variants in the *SPAK* gene have been identified as HTN susceptibility factors in the general population, suggesting that altered WNK–SPAK signaling plays an important role in essential HTN. Here, we highlight recent breakthroughs in this emerging field and discuss areas of consensus and uncertainty.

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Hypertension (HTN) is a substantial public health problem, affecting over a billion people on the planet. It is a major independent risk factor for myocardial infarction, stroke, and end-stage renal disease. The pathogenesis of essential HTN remains unknown, but epidemiological studies point to complex genetic and environmental factors. Genes have a major role in HTN susceptibility, with the heritability of blood pressure (BP) levels estimated to be 30–35%. Major environmental triggers include obesity and diet, especially high Na^+ and low K^+ dietary intake. The recent discovery of Ste20-related proline–alanine-rich kinase (SPAK, also known as serine threonine kinase 39 or STK39) as a HTN susceptibility gene in the general population,[1] together with the known involvement of with-no-lysine (WNK) kinases in a rare familial disorder of HTN and hyperkalemia, now casts light on the potential importance of a multi-gene kinase network in the genesis of HTN. In fact, a flood of recent studies are beginning to shed light on the mechanisms by which dietary salt intake influences renal Na^+ transport and how mutations in these kinases and ion transport genes may lead to BP dysregulation. The objective of this minireview is to highlight recent breakthroughs and to discuss areas of consensus and uncertainty in this emerging field.

THE ROLE OF THE KIDNEY IN HTN

Physiologists have long appreciated the central role of renal salt excretion in the control of BP.[2] Maintenance of a constant intravascular fluid volume and BP depends on the ability of the kidneys to regulate urinary Na^+ excretion. The concerted action of several parallel Na^+ transport mechanisms allows urinary Na^+ excretion to match a wide range of Na^+ dietary intakes, while minimizing fluctuations in the intravascular fluid volume and arterial BP. Although the abnormal function of any one renal salt transport mechanism can usually be compensated for, a greater than normal increase in arterial BP must occur in order for urinary Na^+ excretion to be brought into balance with an increased Na^+ intake. Guyton[2] coined the term for this change in BP as the 'set-point' for controlling Na^+ excretion.

GENETIC EVIDENCE LINKING RENAL SALT HANDLING TO BP REGULATION

Mutations in ~20 different genes have been linked with known single-gene forms of hereditary HTN or hypotension.[3] Astonishingly, all of these genes encode molecules that control the ability of the kidney to maintain salt balance, reiterating the importance of this physiological pathway in regulating BP. The distal Na^+ channel (epithelial sodium channel (ENaC)) and the thiazide-sensitive NaCl cotransporter (NCC) have a central role in BP regulation. Loss-of-function mutations in ENaC can cause the hypotension of pseudohypoaldosteronism (PHA) type I, while gain-of-function mutations result in HTN (Liddle's syndrome). Loss-of-function mutations in NCC cause Gitelman's disease,[4] an autosomal recessive salt-losing nephropathy characterized by hypotension, hypokalemic metabolic alkalosis, and altered divalent cation homeostasis. Conversely, HTN in PHA type II (Gordon's syndrome) ultimately results from a gain-of-function by NCC. This rare autosomal dominant disease of HTN and hyperkalemia results from mutations in two WNK genes, *WNK1* and *WNK4*.[5] In the general population, rare heterozygous loss-of-function mutations in NCC produce clinically significant reductions in BP and protection from HTN.[6] Moreover, studies from the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial, the largest clinical BP-lowering trial in history, clearly demonstrated the morbidity- and mortality-lowering benefits of inhibiting NCC in the treatment of essential HTN.[7] Together with recent genome-wide association studies identifying an important kinase regulator of renal salt transport, SPAK, as a HTN susceptibility gene,[1] it seems likely that essential HTN may be born out of a combination of common and rare susceptibility alleles that affect the ability of the kidney to appropriately respond to dietary salt.

SENSITIVITY TO DIETARY Na^+ AND K^+

As predicted by the Guyton model, dietary salt (NaCl) loading is well established to increase BP in humans. The best evidence comes from tightly controlled, dose–response trials in large populations. For example, the Dietary Approaches to Stop HTN trial convincingly revealed a dose-dependent decrease in BP in response to Na^+ reduction regardless of whether the subjects were hypertensive or nonhypertensive.[8] It should be pointed out that the reduction in BP is heterogenous,[9] with individuals exhibiting a continuous spectrum of responses to a reduction in dietary Na^+. In general, the effect of Na^+ on BP is greatest in Blacks and in individuals with chronic kidney disease or HTN. Thus, it is not just dietary Na^+ that determines BP, but genetic and other factors also have an important impact.[10] One of the most important of these other factors is dietary K^+.[11] High dietary K^+ intake is associated with reduced BP. Furthermore, the rise in BP for a given increase in Na^+ intake is blunted in the setting of a high K^+ intake. For these reasons, recommended dietary regimens to reduce HTN, such as that recommended by the Dietary Approaches to Stop HTN trial, provide a relatively high level of K^+.[10]

The scientific basis for the response to K^+ is not understood. However, the discovery that the WNK–SPAK kinase network regulates renal Na^+ and K^+ transport provides a clue that the impact of K^+ diet on BP might be due to a physiological switch that alters the response of the kidney to either conserve Na^+ or excrete K^+, depending on whether secretion of the mineralocorticoid hormone, aldosterone, is induced by a change in dietary Na^+ or K^+. How renal Na^+ reabsorption and K^+ excretion are coordinately regulated has long been a puzzle because aldosterone controls both processes. In states of intravascular volume contraction (for example, low-Na^+ diet), angiotensin II (AngII) stimulates aldosterone release to maximize renal NaCl reabsorption and restore BP. On the other hand, when aldosterone is released in response to a rise in plasma K^+ (for example, high-K^+ diet), it stimulates maximum K^+ excretion without major effects on renal Na^+ handling.

Studies on how pseudohypoaldosteronism II (PHAII) causes both HTN and hyperkalemia have cast light on how the kidney responds appropriately to aldosterone. Patients with the disease exhibit both excessive Na^+ retention and impaired K^+ excretion despite normal aldosterone and otherwise normal renal function. The discovery that mutations in *WNK1* or *WNK4* cause PHAII[5] revealed a signaling system that may switch the balance between Na^+ reabsorption and K^+ excretion. In PHAII, mutations appear to aberrantly switch the kinases into the 'hypovolemia' position and divorce the system from physiological control, causing deleterious Na^+ and K^+ retention regardless of volume or K^+ status. Although concepts about WNK signaling in the kidney are still emerging, present evidence suggests that the WNK kinases converge with the SPAK kinase to switch the mineralocorticoid hormone response of the kidney to be either Na^+ retaining (antinatriuretic) or K^+ excreting (kaliuretic). This likely occurs because the kinase pathway differently regulates the thiazide-sensitive Na^+ chloride cotransporter, NCC,[12] and the K^+ excretory channel, ROMK (Renal Outer Medullary K channel, Kir 1.1)[13] (Figure 1).

REGULATION OF NCC BY THE WNK–SPAK SWITCH

NCC is the principal determinant of renal NaCl reabsorption in the distal convoluted tubule (DCT).[14] It is tightly regulated by aldosterone and AngII, allowing Na^+ reabsorption in the DCT to be modulated in accord with the demands of salt balance and intravascular pressure. Multiple studies suggest that members of the WNK kinase family converge with SPAK or possibly the related kinase, oxidative stress response kinase 1 (OSR1), to transmit and integrate the aldosterone and AngII response by controlling NCC transport activity.[15,16] Work in model systems and genetically engineered mouse models revealed that wild-type *WNK4* has the capacity to reduce NCC expression at the plasmalemma. As PHAII missense mutations in *WNK4* ($WNK4^{PHAII}$) block this inhibitory activity,[17,18] the apical expression and activity of the transporter are considered to increase in the disease.

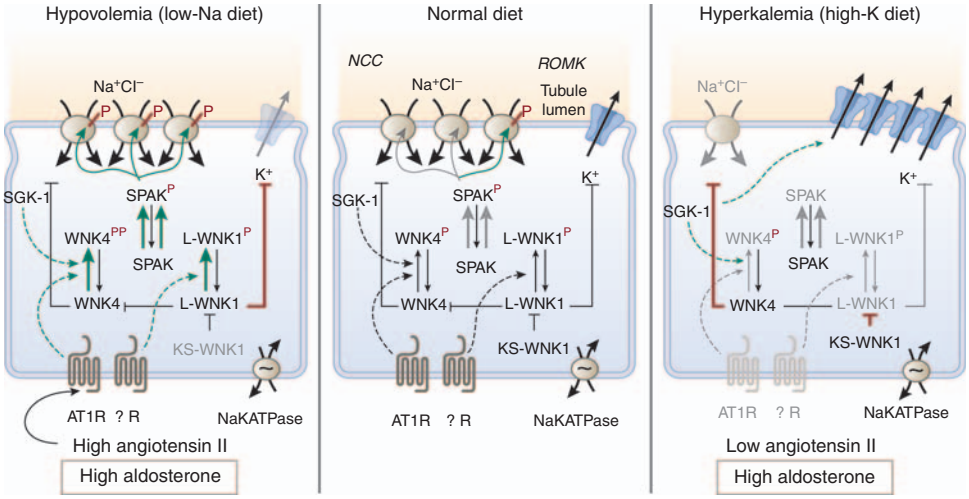

Figure 1 | Model of the WNK–SPAK signal transduction system in the distal nephron, switching the aldosterone response of the kidney to be antinatriuretic (left) or kaliuretic (right). Green arrowheads (activating pathways), red blunt end (inhibitory pathway). The left panel shows the pathway in the setting of a low-Na^+ diet, when AngII and SGK-1 signaling leads to phosphorylation of *WNK4*. This stimulates phosphorylation of SPAK, which, in turn, phosphorylates NCC, activating Na^+ transport to enhance conservation of Na^+ in hypovolemia. Stimulation of unknown receptors is suspected to cause phosphorylation of *L-WNK1*, which can also stimulate SPAK phosphorylation. *L-WNK1* has other functions: (a) It blocks the NCC-inhibitory form of *WNK4*. (b) It inhibits secretion of K^+ via ROMK channels, so as to conserve K^+ despite high aldosterone levels. The right panel shows the pathway in the setting of high dietary K^+ intake, when aldosterone is stimulated and AngII is low. In the absence of sufficient AngII, AT1R cannot activate *WNK4*. This reduces SPAK activation and NCC phosphorylation. At the same time, dietary potassium loading increases the level of *KS-WNK1* isoform to suppress the activity of *L-WNK1*. Consequently, the full inhibitory power of *WNK4* on NCC becomes unleashed, blocking traffic of NCC to the apical membrane and thereby reducing NCC surface density. *KS-WNK1* also blocks the effect of *L-WNK1* on ROMK endocytosis, causing ROMK to increase at the apical membrane. In this way, K^+ secretion in the DCT and connecting tubule/cortical collecting duct is maximized, whereas NCC is suppressed. Aldosterone stimulation of ENaC (not shown) offsets the decreased Na^+ reabsorption by NCC, allowing robust potassium secretion without changes in sodium balance. (*WNK3*, which is believed to antagonize the inhibitory effects of *WNK4* on NCC, and the possible effects of *WNK4* on ROMK are not shown for clarity.) DCT, distal convoluted tubule; NCC, NaCl cotransporter; ROMK, Renal Outer Medullary K channel, Kir 1.1.

Moreover, a $WNK4^{PHAII}$ mouse model shows stimulation of SPAK/OSR1-dependent phosphorylation of NCC to enhance transport activity.[19] Both factors—increased apical membrane expression and increased activity of NCC—likely explain Na^+ retention in PHAII. The effects of *WNK4* on ROMK are somewhat more controversial[20] (also see below), but early work in model systems indicated that $WNK4^{PHAII}$ mutations affect ROMK in an opposite manner to that of NCC. Consequently, Kahle *et al.*[21] postulated that *WNK4* might physiologically shuttle between two states. They suggested that if *WNK4* switched from its basal state to a mode that is mimicked by $WNK4^{PHAII}$, it would tune the aldosterone response such that normally it restores intravascular volume without perturbing K^+ balance.

The physiological mediators of the putative *WNK4* switch have only begun to be identified, but recent work highlights the importance of AngII. As AngII levels are high in the hyperaldosterone state of hypovolemia (or low-salt diet) but are low in hyperkalemia, signaling through the AngII receptor, AT1R, has been a chief suspect in switching *WNK4* into a mode that simultaneously activates NCC and inhibits ROMK. Consistent with this idea, AngII stimulates NCC[22] and inhibits ROMK *in vivo*.[23] Exciting recent studies reveal that AngII stimulation of NCC can be reconstituted in Xenopus oocytes when *WNK4* and SPAK are included in the system.[24] Together with observations that AngII signaling increases phosphorylation of key sites on SPAK and NCC in a *WNK4*-dependent manner, it is likely that *WNK4*–SPAK is the chief arbiter of the signaling pathway between AT1R and NCC. According to this model, activation of the AT1R pathway converts *WNK4* from an inhibiting mode to an NCC-activating form (Figure 1). The activating form of *WNK4* induces a phosphorylation cascade, whereby *WNK4* phosphorylates SPAK and then phospho-SPAK phosphorylates and activates NCC. The stimulatory effects of mutant $WNK4^{PHAII}$ are not augmented by AngII, consistent with the mutations having a gain-of-function effect resulting in constitutive activation of the signaling pathway.

Unlike *WNK4*, *WNK1* is not able to mediate the AT1R signaling response.[24] However, under certain conditions, such as osmotic stress, *WNK1* can phosphorylate and activate SPAK.[25,26] On the basis of these observations, it seems reasonable to hypothesize that a *WNK1*-dependent SPAK activation pathway may also be involved in stimulating NCC under certain physiological settings. Work in cell culture systems indicates the involvement of the PKB/PI3-kinase signaling pathway in activating *WNK1*.[27,28] Such a pathway may be responsible for linking insulin to the regulation of NCC,[29] but thus far suitable studies rigorously connecting these observations are lacking. To date, attention has largely focused on the ability of *WNK1* isoforms to regulate NCC through their modulation of *WNK4* (see below).

In fact, *WNK4* appears be a focal point of regulation by other kinases as well.[30–33] *WNK4* has been shown to form heteroligomeric complexes with *WNK3* and *L-WNK1*.[31,34] Functionally, *WNK3* enhances the transport activity of NCC[31,35] through a SPAK-independent phosphorylation mechanism,[36] whereas *L-WNK1* has antagonistic effects on the NCC-inhibitory form of *WNK4* (see below). Such interactions between different WNKs have been suggested to allow the signal transduction pathway to be precisely tuned to meet the different physiological demands of salt balance.[31] Moreover, recent studies in heterologous expression systems and knockout mice indicate that the aldosterone-induced kinase, SGK-1, modulates *WNK4* activity so as to increase NCC abundance, phosphorylation, and activity.[30,32,37] Two SGK phosphorylation sites have been identified in the C-terminal of *WNK4*.[30,32] As studied in the oocyte expression system, phosphorylation of *WNK4* at these sites blocks the ability of *WNK4* to inhibit NCC.[32] Importantly, C-terminal phosphorylation of *WNK4* does not mimic the stimulatory effects of AngII.[24] Instead, the AngII signaling pathway appears to stimulate the *WNK4* kinase activity needed to phosphorylate SPAK.[38] The precise mechanism by which AngII turns on *WNK4* is unknown, but is likely to involve phosphorylation of the T-loop site in the *WNK4* kinase domain. The dual requirement for both SGK-1 and ATR1 signaling to switch *WNK4* from being an inhibitor to an activator of NCC would provide a mechanism to integrate the effects of high aldosterone and AngII for appropriate activation of NCC in hypovolemia. It would also explain why high aldosterone and SGK activation induced by a high-K diet does not stimulate NCC transport, in which case AngII is not elevated (see below).

It should be pointed out that an important detail of the model has recently been called into question. On the basis of the finding that NCC protein abundance is not augmented in genetically modified *WNK4* mice, which bear a targeted deletion of exons 7–8, it has been argued that *WNK4* may not usually function as an inhibitor of NCC *in vivo*.[39] In fact, because these mice show reduced NCC phosphorylation and a reduced renal Na^+ reabsorptive capacity, a case has been made for the idea that *WNK4* only operates as an activator of NCC *in vivo*. According to this view, $WNK4^{PHAII}$ mutations only enhance the normal activity of the kinase.

Although these new studies with the so-called hypomorphic *WNK4* mice are very interesting and should not be dismissed, they are difficult to square with numerous reports that *WNK4* can inhibit NCC.[17,18,24,34,40,41] Even a modest overexpression of two extra WT *WNK4* transgenes leads to a dramatic decrease in NCC *in vivo*.[42] Furthermore, RNA interference-mediated knockdown of endogenous *WNK4* in human embryonic kidney cells increases NCC.[43] In our opinion, the switch model shown in Figure 1 provides a way to reconcile the different findings, but several key issues must be carefully examined. The major one is to test what function is carried out by exons 7 and 8. It is conceivable, for example, that this region of the kinase is not required for the inhibitory effects of *WNK4*. The switch model points to another possibility. To date, only the NCC activation limb—low-salt diet—has been tested in *WNK4* hypomorphic mice. It will be important to study whether these mice also lose their ability to downregulate NCC in physiological settings such as in hyperkalemia[37] as the model predicts.

WNK REGULATION OF K^+ BALANCE

The physiological uncoupling of aldosterone-dependent K^+ secretion from Na^+ reabsorption has been traditionally explained by the Na^+ and flow-dependent nature of K^+ excretion. In states of intravascular volume depletion, for example, stimulation of NCC at the DCT should diminish the flow and supply of Na^+ that can be delivered downstream to the connecting tubule and cortical collecting duct for efficient Na^+/K^+ exchange, mediated by the epithelial Na^+ channel, ENaC, and the K^+ secretory channels, ROMK and BK.[44] In states of antidiuresis, antidiuretic hormone stimulates ROMK to maintain potassium secretion in spite of the decrease in distal flow. However, the textbook explanation is not completely satisfactory. It does not adequately explain how the kidney stimulates maximum K^+ excretion without major effects on renal Na^+ handling when aldosterone is elevated by increases in plasma K^+. The classic teaching also overlooks that a significant fraction of K^+ secretion likely occurs in the late DCT, especially when animals are fed a high-K^+ diet. In fact, a K^+-secretory pathway that is not dependent on ENaC has recently been described.[45] Accumulating evidence favors the idea that a WNK signaling pathway may operate in parallel with the classic factors to achieve a robust kaliuretic response to a K^+ load without altering Na^+ balance.

The signaling response to K^+ is just beginning to be unraveled. *L-WNK1*, *WNK3*, and *WNK4* all downregulate ROMK at the cell surface in heterologous expression systems, likely by stimulating clathrin-dependent endocytosis. But, so far, only one *WNK* gene has emerged as a significant player *in vivo*. Studies in genetically modified *WNK4* mice,[19,42] for example, have been interpreted to indicate that *WNK4* may not have a role in the physiological regulation of ROMK, but further studies with better, more specific ROMK antibodies and definitive tests of ROMK function (for example, patch-clamp analysis and measurements of potassium secretion in preparations such as the isolated perfused tubule, in which confounding variables can be tightly controlled) are desperately required to test this hypothesis rigorously. *WNK3* is not associated with PHAII and its physiological relevance in K^+ balance has not been studied. By contrast, accumulating evidence indicates that products of the *WNK1* gene are likely to operate as a key arbiter of the switch response to K^+ diet.

Alternative promoter usage of the *WNK1* gene produces a kidney-specific short form of *WNK1*,[46,47] called *KS-WNK1*, and a more ubiquitous long form, called *L-WNK1*. Unlike *L-WNK1*, the kinase-deficient *KS-WNK1* form has no inhibitory effect on ROMK.[48,49] Instead, *KS-WNK1* negatively

modulates *L-WNK1* to suppress ROMK channel endocytosis. In fact, the apical surface expression of ROMK in the distal nephron is enhanced in transgenic mice that overexpress *KS-WNK1*.[50] Most importantly, the relative abundance of the *WNK1* isoforms is regulated by dietary K^+. As acute dietary K^+ loading increases the relative abundance of *KS-WNK1*, while dietary K^+ restriction increases the relative abundance of *L-WNK1*, the *WNK1* isoform switch is well positioned to serve an important role in the physiological regulation of ROMK apical surface density and K^+ balance.[48,49]

Importantly, the two *WNK1* isoforms regulate NCC in a manner different from that of ROMK, providing a mechanism to maintain Na^+ balance in the high-aldosterone state of dietary K^+ loading. Heterologous expression studies reveal that *L-WNK1* upregulates NCC by blocking the inhibitory form of *WNK4*. *KS-WNK1*, on the other hand, antagonizes the effects of *L-WNK1* and therefore indirectly inhibits NCC.[40] Consequently, the full inhibitory power of *WNK4* on NCC may become unleashed in states of dietary K^+ loading, when *KS-WNK1* expression is augmented. As the high-aldosterone state associated with a high-K diet stimulates SGK-1, the latter would act on ROMK to promote K secretion.[30,51] However, in the absence of AngII, the SGK phosphorylation of C-terminal sites described above would be insufficient to switch *WNK4* into its kinase-active form necessary for SPAK activation and NCC phosphorylation. The functional significance of downregulation of NCC in the hyperaldosterone state of high dietary K^+ together with inhibition of Na^+ transport in the thick ascending limb[52] would be to counterbalance the enhanced ENaC-dependent Na^+ reabsorption in the connecting tubule and cortical collecting duct, and thereby preserve Na^+ homeostasis. Thus, a main prediction of the linkage between K^+ and Na^+ balance is based on whether transport by NCC is downregulated in response to dietary K^+ loading as illustrated in Figure 1. Recent studies have indeed shown that the expression and phosphorylation of NCC are reduced by a high-K^+ diet.[37]

Recent studies reveal a potential mechanism to explain how WNKs regulate NCC and ROMK differently, involving modulation of two distinct trafficking pathways. ROMK contains an unusual variant of the 'NPXY' internalization signal, which serves as a docking site for the clathrin adaptor molecule, ARH (autosomal recessive hypercholesterolemia).[53] *WNK1* stimulates ROMK endocytosis through an ARH-dependent pathway. Unlike ROMK, NCC does not contain NPXY-type signals and, therefore, does not have the capacity to engage ARH. In fact, Subramanya *et al.*[43] recently discovered that *WNK4* does not stimulate NCC endocytosis. Instead, *WNK4* acts on newly synthesized NCC in the secretory pathway to stimulate interaction with the AP-3 clathrin adaptor and divert the transporter into the lysosomal pathway.

SPAK REGULATION

SPAK was identified as a membrane-associated kinase that interacts with cation-chloride cotransporters and WNKs.[54] Since this discovery in 2002, work in expression systems and model organisms has clearly established that SPAK directly regulates members of the electroneutral cation-chloride cotransporter gene family, including NCC, as a part of an evolutionarily conserved signaling pathway that is important for controlling electroneutral Na^+ transport and osmotic cell volume regulation.[55] The discovery of SPAK as a HTN susceptibility gene highlights its potential role in the regulation of renal Na^+ transport for the control of BP. On the basis of recent studies, a working model for NCC activation in the kidney (Figure 1) envisages an upstream WNK (either *WNK4* or *WNK1*) phosphorylating and activating SPAK, which in turn phosphorylates and activates the cotransporter. The model is largely based on biochemical studies in expression systems showing that *WNK1* or *WNK4* directly interacts with SPAK and phosphorylates residues T243 and S383 (mouse numbering).[38] Although the functional role of S383 phosphorylation is still not known, phosphorylation of T243 is absolutely required for SPAK activation.[56] Furthermore, activation of the *WNK4*–SPAK signaling system in DCT cells *in vitro* by hypotonic stress or AngII induces phosphorylation of NCC.[24] Obviously, future studies are required to test the validity of this model *in vivo*.

The exclusivity of SPAK in the proposed model has not been established. SPAK is closely related to OSR1, which, similar to SPAK, is widely expressed in many tissues, including the kidney.[57] SPAK is believed to have evolved from OSR1 by gene duplication.[55] OSR1 can also be activated by WNKs and can phosphorylate NCC.[25,26] Although it might be imagined that OSR1 could substitute for SPAK, no change in OSR1 expression is found in SPAK-null animals or when SPAK is knocked down by RNA interference.[58] Moreover, eliminating SPAK from cells that normally express both SPAK and OSR1 results in a distinct impairment of cotransporter function. Thus, SPAK and OSR1 are regulated independently and may have distinct functional roles, although the exact nature of their roles remains to be defined. In addition, there are reports that signaling via PKC isotypes can activate SPAK in other systems.[59,60] Although such a role in the kidney has not been evaluated, it is possible that renal SPAK activity is also regulated via non-WNK pathways.

FUTURE STUDIES

Although the scheme described in Figure 1 synthesizes much of the currently available data to present a unified mechanism for SPAK–WNK control of Na^+–K^+ balance and BP, we consider it as an evolving model and as only a framework for guiding future investigation. Certainly, much of the model needs to be rigorously tested, and much will be learned in the process. In our opinion, a convergence of powerful approaches addressing this problem can promise to rapidly yield a comprehensive and clinically useful understanding of multigene kinase control of BP in health and disease.

Central to this advance will be genetically modified mouse models that either eliminate or modify kinases of interest.

Despite being a critical and powerful approach, such studies need to be interpreted carefully, as the kidney has a strong ability to compensate for defective or missing components. Thus, testing the physiological validity of findings from mouse models will require carefully performed parallel studies in normal animals. Evaluation of the effects of altered Na^+ and K^+ diet on the status of switch elements together with measurements of Na^+ and K^+ transport *in vivo* and in isolated perfused tubules should be particularly informative.

Although carefully performed animal studies are necessary, important, and insightful, they may not offer detailed insights into the underlying cellular, biochemical, and molecular mechanisms. An exhaustive mechanistic analysis will require further investigation in model systems, including oocytes and cultured cells. For such studies, it will be critical to complement and validate the standard kinase overexpression experiments with RNA interference-mediated knockdown approaches. It should be particularly instructive, for example, to replace endogenous components of the signaling pathway with physiological doses of strategically designed mutants. Vigilant monitoring of the subcellular localization and phosphorylation status of the relevant parts of the signaling network will be crucial to guide interpretation.

Continued genetic analysis in humans promises to be a crucial arm of a multidisciplinary investigation, serving to identify additional variants that influence BP. This approach has been highly successful for rare alleles, but association findings for common alleles have been much less consistent. Earlier genome-wide SNP arrays offered uneven and incomplete coverage of variants in genes in this network, particularly *WNK4*.[61] Studies that focus on a single gene or on a few genes often consider different SNPs, making cross-validation a challenge. However, the tide is rapidly turning. Currently, newer arrays offer more complete coverage. Data from multiple studies can be more easily combined ($>100{,}000$ total subjects) or filled in through imputation, making it likely that the next generation of genome-wide association studies will yield additional insights.[62,63] With the application of new high-throughput approaches to identify rare variants, assess copy number, and look for epigenetic variables such as DNA methylation, the field should soon have a more comprehensive view of the genetic basis of BP.

Each of these powerful approaches has both strengths and weaknesses, but taken together, they should continue to rapidly advance our understanding of the mechanisms underlying renal control of BP.

DISCLOSURE

All the authors declared no competing interests.

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